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Research Article

Metabarcoding outperforms traditional electrofishing in decapod and fish inventories, paving the way for enhanced biodiversity monitoring in the Caribbean

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Abstract

Environmental DNA (eDNA) metabarcoding revolutionized the biodiversity monitoring in aquatic ecosystems, giving access to taxonomic lists in a non-disruptive way. Although the method has limits, such as reduced taxonomic resolution for certain groups and difficulties in estimating species abundance, it has proven its effectiveness in many contexts. In Martinique, a Caribbean island, traditional methods like electrofishing (TEF) are known to be stressful for organisms, non-selective and disruptive for the ecosystem, and have been progressively abandoned for routine monitoring. The aim of this project was to explore the possibility of using the eDNA-based metabarcoding method for the detection of fish and decapods in Martinique streams, by first validating it with TEF. We selected 14 stations, a representative panel of the river diversity, and performed TEF and eDNA-based monitoring to compare both, based on the species richness. Then, from eDNA taxonomic inventories, we assessed the ecological state of the studied stations, using Simpson index and investigated how stations abiotic characteristics shape assemblages. Here, we confirmed the eDNA metabarcoding method is a reliable tool for monitoring fish and decapods, confirming most of the taxa caught by TEF and revealing the presence of additional (native and/or invasive) species. We faced some issues in discriminating some genetically close species (e.g. *Sicydium* sp.) potentially leading to under-representation in community assemblages, but not in functional diversity. Additional efforts are needed to raise standardized protocols, but we encourage stakeholders to join such an initiative to shed light on the rich biodiversity in sometimes poorly studied regions and to face invasions.

Key words: biodiversity hotspot, ecological assessment, environmental DNA, Martinique island, method validation

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Introduction

Freshwater ecosystems are of high importance, providing habitat for at least 6% of known species (and probably many more to discover) on < 0.8% of the total Earth surface (Michelet 2017). Nevertheless, unsustainable human activity (i.e. use of pesticides, urbanization, dredging and draining, for example) has had a considerable impact on these freshwater ecosystems in recent years, with an estimated loss of 84% of the biodiversity since 1970 and almost one species out of three threatened with extinction, all taxa combined (Magurran 2009; WWF 2020). These losses weaken the environment by affecting the distribution and composition of native communities, sometimes disrupting migratory patterns and associated life cycles (Magurran 2009; Engman and Ramirez 2012). Tropical islands are particularly vulnerable, characterized by low species diversity combined to high endemism (Nivet et al. 2010) on small areas (Myers et al. 2000). For instance, Martinique is a rugged island, located in Lesser Antilles archipelago, presenting a wide variety of landscapes and terrestrial ecosystems with more than 70 permanent rivers (as well as many non-permanent ones and tropical forests wetlands), justifying its place in one the 25 hotspots of biodiversity (Anadón-Irizarry et al. 2012). These disruptions also make the ecological niche much more permeable to invaders, introduced mainly via aquaristic activities and aquaculture, one of the major factors in current biodiversity loss (Gherardi et al. 2008; Nunes et al. 2015; Rodríguez-Barreras et al. 2020). For all these reasons, there is an urgent need for conservation of these freshwater ecosystems, based on environmental protection programs (Flitcroft et al. 2019). However, these programs require a good knowledge of the environment and the distribution of native and native species, which calls for inventories with accurate species identification and assessment (endemic, rare, endangered or invasive).

Biological inventories of macro-organisms in aquatic environments were initially based on traditional methods like direct capture, electrofishing, or baited traps, known to be non-selective, time-consuming and particularly disruptive for the environment (Hänfling et al. 2016; Wang et al. 2021), making them increasingly controversial. Traditional electrofishing (TEF) for example, which is largely used in freshwater environment, uses electric currents to temporarily stun fish, making them easier to capture and study (Pusey et al. 1998). While effective, this method can be stressful or even harmful to the organisms and potentially disruptive to the fragile ecosystems, harboring small populations, which researchers seek to understand and protect (Snyder 2003; Dolan and Miranda 2004). Finally, the labor-intensive and time-consuming nature of these inventories limits the scale of their use, especially in larger or more remote areas (Hense et al. 2010; Evans et al. 2017). Despite these drawbacks, traditional methods remain invaluable for certain research aims, particularly when precise biometric data or population densities are needed (Thomsen and Willerslev 2015; Evans et al. 2017).

In recent years, freshwater inventories have undergone a revolution with the emergence of monitoring techniques based on the detection of DNA shed by organisms in the water (i.e. skin cells, mucus or feces) dubbed 'environmental DNA' (Ficetola et al. 2008). This approach offers the possibility to detect targeted species even with low population density (i.e. invasive and/or rare, endangered endemic species) without the need to observe it, at any stage of

life, regardless of their size or ecology (i.e. very small or even microscopic, and sometimes cryptic, living underground or in disconnected ditches) (Ficetola et al. 2008; Thomsen and Willerslev 2015). The eDNA-based method rapidly gained traction in the field of biodiversity assessments, representing a promising alternative or complement to traditional methods, thanks to its low disruptiveness, the ease of implementing it on field at large scale and its ability to detect a broad spectrum of species when using metabarcoding (Valentini et al. 2016; Pont et al. 2018; Taberlet et al. 2018). However, while eDNA metabarcoding presents significant opportunities, it is not without challenges and drawbacks. First, there is a need for a resolutive barcode to discriminate taxa. For instance, MiFish, Teleo and 12S-V5 primers (designed respectively by Riaz et al. 2011; Miya et al. 2015; Valentini et al. 2016) all target a quite short fragment (65 - 175 bp) in a highly conserved genetic region (12S rRNA) and mlCOIintF/jgHCO2198 primers (Leray et al. 2013) target a 313 bp fragment of the cytochrome oxidase sub-unit I (COI). Then, robust reference databases are essential, containing at least genetic sequences for all species within the study area, and additionally, species susceptible to be introduced (Schenekar et al. 2020; Marques et al. 2021). Such reference databases, coupled with a good knowledge on the studied environment and its native communities, are essential for comprehensive and accurate biodiversity assessments (Schenekar et al. 2020; Marques et al. 2021). Moreover, eDNA-based methods now reached high sensitivity yields and controls samples (i.e. for cross-station or lab contaminations) are therefore crucial, in addition to rigorous protocols, to ensure sample integrity, optimization of species discrimination and avoidance of false positives.

As eDNA-based methods grow in popularity regarding their operationality for biodiversity assessment, they are more and more integrated into legal monitoring frameworks and decision making (Morisette et al. 2021; Adams et al. 2024; Kelly et al. 2024). Validation through comparisons with traditional methods is crucial to ensure the reliability of eDNA in biodiversity assessments. While eDNA metabarcoding has many advantages, it should complement, not replace, traditional approaches, which provide key context on species abundance, age structure, and health (Evans et al. 2017). By integrating eDNA with traditional techniques, researchers can achieve a more holistic understanding of ecosystems. For instance, eDNA can be used for rapid initial surveys to identify species presence across large areas, while traditional methods can be employed for more detailed studies in key locations (Evans et al. 2017; Baudry et al. 2023). This combined approach allows for both broad-scale biodiversity assessments and in-depth investigations of particular species or habitats.

In this study, we investigated the potential of eDNA metabarcoding for freshwater fish and decapods' long-term monitoring in Martinique, using respectively MiFish (Miya et al. 2015) and MiDeca (Komai et al. 2019) primers. We compared species richness and taxonomic diversity revealed by both TEF and eDNA-based method, across 14 stations selected to ensure representative coverage of all the island's rivers. We hypothesized that eDNA-based metabarcoding method would reveal species richness similar to - or higher –than TEF, especially by detecting rare, cryptic or elusive taxa. We also expected the Simpson index to be a meaningful indicator of ecological quality, reflecting patterns of species dominance and community homogenization, for instance, in southern sites where we expected lower diversity and a stronger dominance of invasive species.

Materials and methods

Study area and sampling sites

Martinique is a Caribbean island of 1128 km2 belonging to Lesser Antilles (14°39'00"N, 61°00'54"W) and dominated by a rainy tropical climate, leading to a vast hydrographic network encompassing 70 main permanent rivers, fed by at least as many tributaries (Baudry et al. 2021). The presence of the Montagne Pelée volcano (1397 m height) in the northern part of the island induces a difference in hydromorphologies, with northern rivers characterized by steep slopes and strong waterflows and inversely, southern rivers being larger and slow-moving.

In this study, we sampled 14 locations, spread over the territory, from the north to the south (Fig. 1), for the most accurate overview of the biodiversity in presence. Eight locations were selected in the northern part (CERon, COULeuvre, CARbet, Maison ROUsse, LORrain, Fonds St-Jacques, BASsignac and GUEs) and six in the southern part of the island (FRançois, SainT-Esprit, Petit BOUrg, LOWinsky, MADeleine and DORmante).

Each station was sampled in April 2023 by both eDNA filtration method (first, to minimize contamination risks) and TEF, following the protocols described below. At each of the stations, the physico-chemical characteristics (pH, temperature, oxygen concentration and conductivity) (Suppl. material 1: S1) were measured using a Hanna® HI98129 instrument.

eDNA sampling

Filtration were performed on-site, before TEF as said above, through 0.45 µm nitrocellulose filters (Sartorius® 47 mm diameter), using a hand-operated vacuum pump (Nalgene™) together with a 1L-filtration unit (Nalgene™), as described in Baudry et al. (2021). Sampling was carried out along a transect starting from the riverbank outward, or in flowing sections, depending on the rivers considered, with two independent eDNA samples taken per station. Each sample was filtered until clogging occurred, typically between one and 2.5 liters per replicate. They were then removed and placed (folded in quarters) into 1.5 mL tubes filled with 1 mL of absolute (99%) molecular-grade ethanol, using sterile forceps.

To avoid potential field cross-contamination, sampling material was decontaminated using 20% bleach and thoroughly rinsed using tap water after each sampling and a blank control sample (1L of distilled water) was done. All eDNA samples were stored in a cooling bag until their return to the laboratory, where they were stored at 4 °C, until eDNA extraction, showing satisfactory yields if processed quickly and ease of use on-field (Renshaw et al. 2015; Majaneva et al. 2018).

Traditional Electro-Fishing (TEF) sampling

The chosen protocol was adapted from Lefrancois et al. (2024), using a Smith-Root LR-24 backpack electrofisher, set on 250V (direct current) or 500V (pulsed current) (10 to 16 Hz and 15 to 25 A, respectively), depending on conductivity and hydrological characteristics of the stations. Briefly, with reasonable human effort (3 or 4 operators), the objective was to detect the maximal fish and crustacean species richness, following past observations (Lim et al. 2002; Baudry et al. 2024), with a minimal habitat disturbance. Each station was surveyed

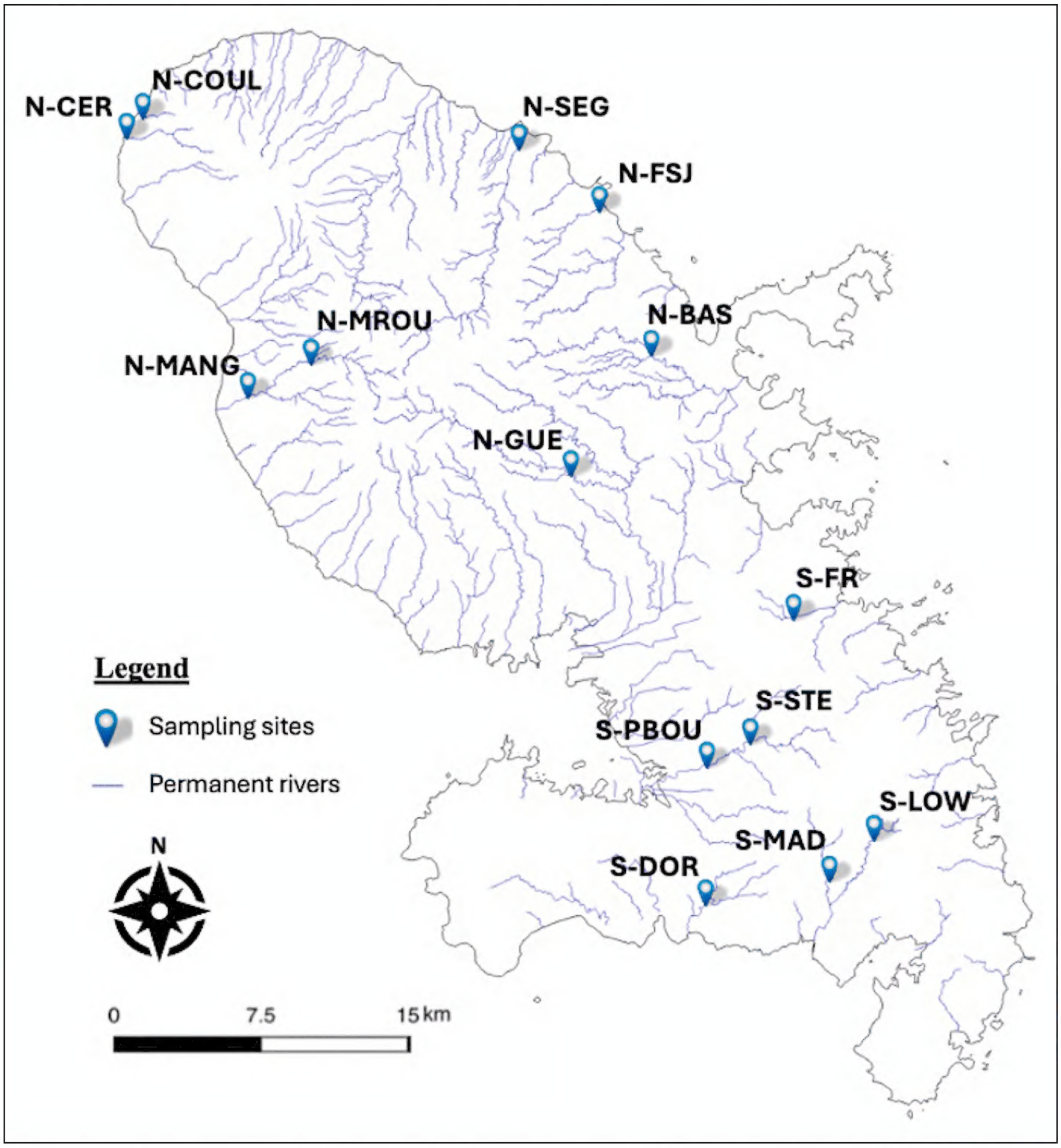

Figure 1. The hydrological network of Martinique, in Lesser Antilles, with the location of the 14 stations sampled during this study, highlighted with their code preceded by their location (N- for northern part and S- for southern part).

using spot-fishing, a method involving short, targeted electrofishing burst (~15 seconds per spot) over discrete areas (~10 m2), and progressing upstream for approximately fifty meters. If all the species known to be present at the station (based on historical data) were not captured, additional spot-fishing was carried out, targeting certain micro-habitats that were favorable to certain species (for example, stumps and banks for eels). Once caught, the specimens (fish and crustaceans) were directly taken back to the riverbank to be identified, sorted and sampled. These operations must be carried out very efficiently, as the water in the tanks heats up quickly in such a tropical climate, which can lead to significant losses. As the aim here was to verify the species' presence (not to quantify), we have therefore chosen to handle the individuals as little as possible, taking

no biometric measurements (except for taxa of interest such as the American eel - *Anguilla rostrata*). Individuals were therefore identified visually, down to species level (or genus level for juvenile individuals). A maximum of 5 individuals per species and per station were non-destructively sampled, by taking mucus or a fin fragment from fish, or by taking a P4 leg fragment from crustaceans. This was intended to, first, confirm the species identification and then, contribute to complete the genetic database (see sections below). The samples were then referenced (station, species and date) and preserved in absolute (99%) molecular-grade ethanol, in 1.5 mL tubes, until the DNA was extracted in the laboratory.

Lab analysis

(e)DNA extraction

DNA (and eDNA) extractions were performed in dedicated rooms, different from that used for PCRs preparations, with benches, tools and surfaces bleach-disinfected before processing samples. From tissue, DNA was extracted using Qiagen DNeasy Blood & Tissue Kit, following manufacturers' guidelines. Concerning the extraction from filters, some minor modifications were applied, following Baudry et al. (2021): ¼ of each filter was cut into small pieces, using sterilized forceps and scissors and dried for thirty minutes (to evaporate the ethanol), into a 2 mL Eppendorf tube. Lysis reagents (450 µL of ATL buffer and 50 µL Proteinase K) were added, submerging the filter fragment, and then vortexed before incubation at 56 °C for 3 hours. The following steps (washing) were done as described by the manufacturer, until the elution, in 60 µL of AE buffer (instead of 200 µL), to concentrate the eDNA.

For both DNA and eDNA, the extraction yields were measured (concentration and absorbance ratios) using the Implen® N60/N50 nanophotometer (Implen GmbH, Munchen, Germany).

Sanger sequencing

First, to ensure the species identity of fish and crustaceans caught, the COI gene was sequenced, using the universal primers FishF1-TCAACCAACCACAAAGACATTGGCAC, FishF2-TCGACTAATCATAAAGATATCGGCAC and FishR1-TAGACTTCTGGGTGGCCAAAGAATCA, FishR2-5'ACTTCAGGGTGACCGAAGAATCAGAA for fish (Ward et al. 2005) and LCO1490-GGTCAACAAATCATAAAGATATTGG and HCO2198-TGATTTTTTGGTCACCCTGAAGTTTA for decapods (Folmer et al. 1994). For each individual, Polymerase Chain Reaction (PCR) was performed following Chucholl et al. (2015) with minor modifications: 2.5 min at 95 °C for initial denaturing, followed by 35 cycles of 45 sec at 95 °C, 1 min at 50 °C and 1 min at 72 °C, and finally 10 min at 72 °C for final elongation. PCR products were purified and 1/10 diluted before sequencing, in both forward and reverse direction, on an Applied Biosystems SeqStudio Genetic Analyzer (Waltham, U.S.A.).

Once the species' identity was verified, DNA extracts were used to complete the genetic database for both fish (12S rRNA) and crustaceans (16S rRNA). The protocol used was the same as described before, with metabarcoding primers MiFish (Miya et al. 2015) and MiDeca (Komai et al. 2019) (see just below).

The sequences obtained (12S rRNA and 16S rRNA) were cleaned and trimmed using Geneious Pro R10 software (https://www.geneious.com; Kearse et al. 2012). They were added to MIDORI2, a newly curated database for eukaryotic taxonomic assignments (Leray et al. 2022), giving a complete database for fish and crustaceans assignments in Martinique (Lim et al. 2002; Baudry et al. 2024). Within this database, an additional curation was done to remove sequences containing ambiguities (N with *maxambig = 0*) or of poor quality (length – *minlength = 150* - or homopolymer – *maxhomop = 10*).

Metabarcoding amplifications

To enable the sequencing of all samples in a single Illumina run, 2-steps PCRs were performed, using MiFish-U-F- GTCGGTAAAACTCGTGCCAGC and MiFish-U-R- CATAGTGGGGTATCTAATCCCAGTTTG primers targeting a 175 bp fragment of the mitochondrial 12S rRNA gene (for fish, Miya et al. 2015) and MiDeca-F- GGACGATAAGACCCTATAAA and MiDeca-R- ACGCTGTTATCCCTAAAGT primers targeting a 164 bp fragment of the mitochondrial 16S rRNA gene (for crustaceans, Komai et al. 2019), including adapters (forward: TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG and reverse: GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG).

PCR reactions were set up in a sterile room, decontaminated every night by UV-light treatment. Each eDNA sample was amplified four times, representing eight PCR reactions per station. PCR reactions were carried out in a 25 µL final volume containing: 12.5 µL of KAPA HiFi HotStart ReadyMix (Roche), 5 µL of each primer with index (final concentration 0.2 µM) and 2.5 µL of template. Each PCR plate contained one negative control (i.e. no-template DNA), to assess for potential contamination during the amplification and three positive mock controls (for each taxa, fish and decapods). The first mock sample corresponds to an equimolar mix of DNA from individuals representing 19 species of decapods and 27 species of fish (Suppl. material 1: S2). In the second and third mocks, DNA were mixed using different quantities to simulate variation of taxa relative abundance. Mock samples were used as positive control during the PCR amplifications' step and were used to calibrate the taxonomic inference from the genetic reference database (see below).

Amplifications programs were: activation at 95 °C for 3 min followed by 35 cycles of 98 °C for 30 sec, 65 °C (60 °C for MiDeca) for 30 sec and 72 °C for 30 sec, and finally 72 °C for 5 min, for final extension. PCR products were visualized on 1.5% agarose gels and then pooled together per station - resulting in two sequencing results per station. They were then sent to PGTB sequencing platform in Bordeaux (France) for quality check (using TapeStation, Agilent, USA), library preparation (2nd PCR) and sequencing on Illumina NextSeq 2000 (U.S.A.), using 2 x 150 pb kit.

Bioinformatics and data analyses

Illumina sequencing handling

Reads generated by Illumina NextSeq 2000 sequencing were handled using DADA2 package (v1.30.0; Callahan et al. 2016) implemented in R (v4.3.2; R Development Core Team, 2023). Primers were removed, reads with Ns were

pre-filtered and quality profiles were inspected. Considering these profiles, especially the quality score in end sequenced reads, and expected lengths, reads were filtered and trimmed. They were then dereplicated and pair-ended merged, using the error model implemented in DADA2. Finally, chimera were removed from the final sequence table and taxonomic assignments (using the curated database) were done using mothur (Schloss et al. 2009) with RDP classifier using the *classify.seqs()* command with *method=wang*, *iters=1000* and *cutoff=75* parameters.

To limit the interpretation of low abundant erroneous DNA reads related to potential contaminants, PCR amplification or sequencing errors, we added additional filtering steps. Amplicons Sequences Variants (ASV) produced by DADA2 pipeline represented by < 10 reads in a sample and then, the ones representing < 0.1% of the total number of reads were removed from the analyses. After these filtering steps, we summed the reads for each ASV at each station and converted these counts into relative proportions by dividing each ASV's read count by the total read count of that station. No filtering criteria based on representativity of taxa in a minimum of replicates per station was used, and all taxa were conserved in the final taxonomic inventory.

Data analyses

All statistical and graphical analyses were performed in the R environment (v4.3.2; R Development Core Team 2023). Before each statistical treatment, when appropriate, data normality and variance homogeneity were verified using Shapiro-Wilk and Bartlett tests, respectively. Maps were generated using QGIS 2.18 (Las Palmas) software (QGIS Team Development 2016): Martinique map was imported from the database ©IGN and the streams from BD Carthage® and BD Topo®.

We first analyzed stations' characteristics and searched for correlations between physico-chemical parameters (altitude, conductivity, pH, temperature, oxygen concentration) depending on the geographical situation of the considered station, based on a principal component analysis (PCA) (FactoMineR and factoextra packages; Lê et al. 2008; Kassambara and Mundt 2020). Contribution of each variable was visualized using *fviz_contrib()* and *fviz_pca_var()* functions and they were then projected on the factorial axes using ggplot2 (Wickham 2016).

For eDNA, after taxonomic assignment of ASVs, fish and decapods taxonomic lists were produced and taxa sorted according to their known occurrence in freshwater or marine environments. For example, *Caranx* sp.– a marine genus resulting from the human consumption – was removed from the dataset here for species richness calculations. Then, we decided to pool all ASVs related to Loricariidae sp. together, as they are genetically and morphologically very close, making their identification difficult. Moreover, many of them are sold for aquarium trade. The influence of the method (TEF vs. eDNA) on those results (species richness) was analyzed based on an analysis of variance (ANOVA), considering a station effect. Species richness was then plotted for each station to visualize those assemblage differences individually.

From eDNA data, Simpson index was calculated using phyloseq package (McMurdie and Holmes 2013) and then community structure comparison between station was tested, for fish, decapods and both mixed, using Bray-Curtis dissimilarity index implemented in phyloseq and ape packages (Paradis and Schliep 2019) and visualized using Non-metric MultiDimensional Scaling (NMDS).

Influence of the exposition (north/south) of the station was assessed using a permutational multivariate analysis of variance (PERMANOVA) using adonis2() from the vegan package (Oksanen et al. 2022). Then, canonical ordination methods were used to investigate the influence of metadata (north/south exposition, temperature, pH, conductivity and oxygen) on species assemblages, using vegan package (Oksanen et al. 2022), which include Redundancy Analysis (RDA) and Canonical Correspondence Analysis (CCA) (Legendre and Legendre 2012). After a gradient length analysis, using detrended correspondence analysis (DCA) (length < 3, suggesting a linear response of taxa to environmental gradients; Lepš and Šmilauer 2003), RDA models were run on Hellinger-transformed ASV abundance data (Legendre and Gallagher 2001). Conductivity, temperature, pH, oxygen concentration, and north/south exposure were used as predictors. Global model significance was assessed via permutation tests (999 permutations), and the contribution of individual variables was evaluated using both sequential (Type I) and marginal (Type III) tests. Finally, the correlation between assemblages' dissimilarity and geographical distances (Suppl. material 1: S3) between each station was tested with a Mantel's test together with a Spearman's rank correlation test, using vegan (Oksanen et al. 2022) and geosphere (Hijmans 2022) packages.

Results

Environmental characterization of sampling sites

Conductivity and temperature were the most influential variables, contributing respectively 30.79% and 28.61% to the variation explained by the axis 1 (Fig. 2A). Inversely, pH played a major role (65.48%) in shaping the axis 2 (Fig. 2A). As expected, temperature and conductivity were negatively correlated with altitude, and oxygen concentration is positively correlated with altitude (Fig. 2B). All stations seemed to exhibit variable pH values, but the north cluster was mainly characterized by lower temperatures and conductivity than the cluster of stations from the south (Fig. 2C).

Bioinformatics and dataset clean-up

In total, for the 14 stations studied (without the mocks), 11,656,029 reads were generated for fish (mean 832,573.5 ± 142,312.1 per station) and 14,813,701 reads for decapods (mean 1,058,121.5 ± 408,818.2 per station). After data filtering, taxonomic assignment and curation, average sample read counts per station was 570,082.9 ± 124,442.6 for fish and 963,464.9 ± 388,726.8 for decapods (Suppl. material 1: S4).

Comparison of TEF vs. eDNA

The number of species captured (TEF) ranged from one to seven for fish and three to seven for decapods, while eDNA-based method detected between four and eleven species of fish and four and nine of decapods (Fig. 3). That said, the eDNA-based method detected significantly more fish species than TEF (7.93 ± 1.94 vs. 4.28 ± 1.68 using TEF; $F = 53.41$, $p < 0.001$) (Fig. 3A) and more decapod species (6.93 ± 1.68 vs. 5.36 ± 1.28 using TEF; $F = 6.47$, $p < 0.05$) (Fig. 3B).

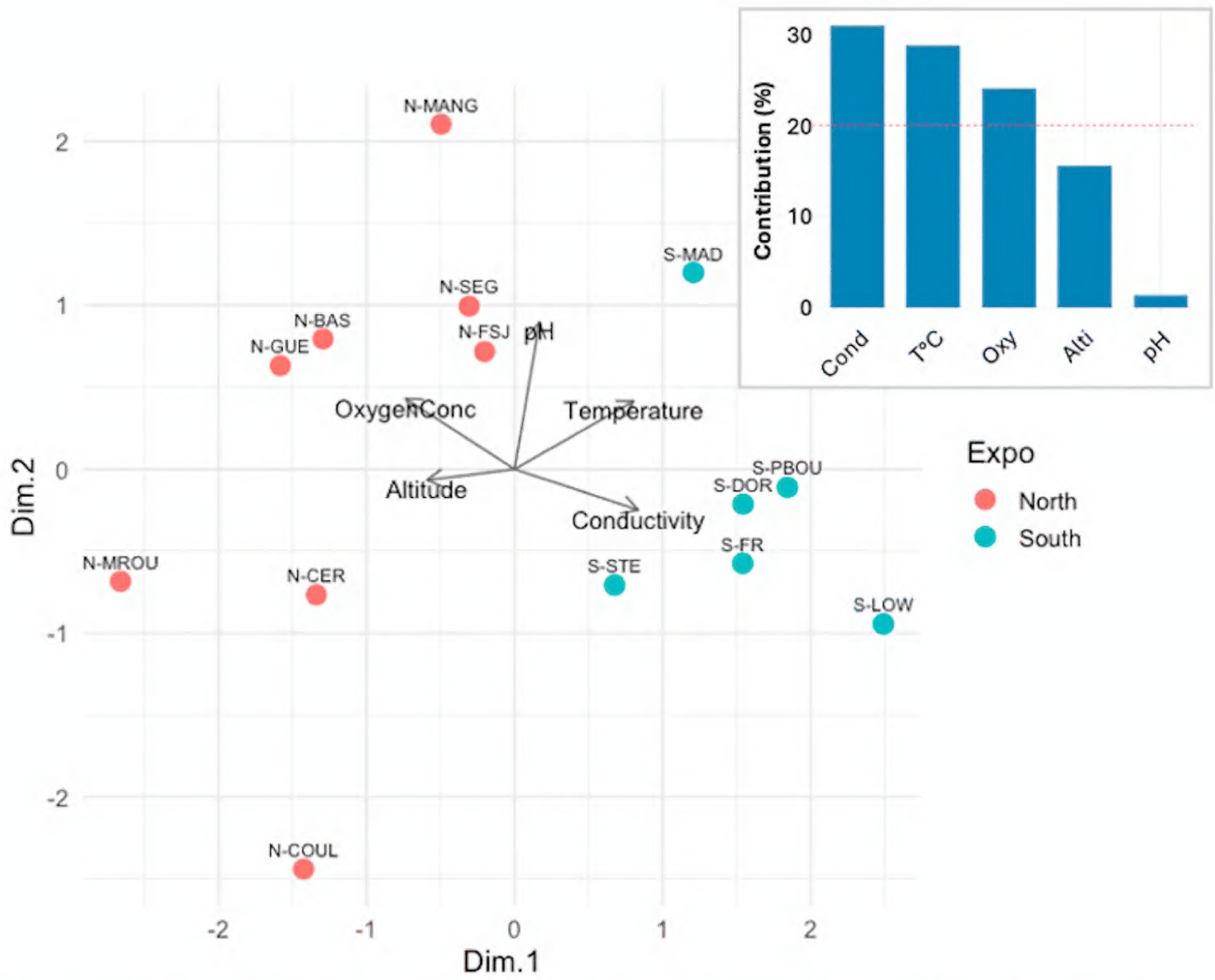

Figure 2. Principal component analysis (PCA) on physico-chemical parameters measured (conductivity, temperature, oxygen concentration, altitude and pH) within the 14 stations studied, related to their exposition, in the northern or southern part of Martinique.

This trend was confirmed when analyzing each station separately, with for example nine fish species detected by eDNA against four by TEF at N-BAS station, or seven species detected by eDNA at S-FR station against only one by TEF (Fig. 3C). TEF reached comparable results at N-MROU station, with four fish species reported with each method (Fig. 3C). Most importantly, in a validation context, eDNA succeeded in detecting all fish species caught by TEF, so sometimes reporting new species presence, not observed on field (Fig. 3C). Just noted the inability of eDNA to discriminate *Sicydium* sp. species (*S. plumieri* and *S. punctatum*), unlike TEF.

For decapods detection, the results were not as clear-cut, with 10 stations (N-BAS, N-CER, N-COUL, N-GUE, S-LOW, S-MAD, N-MANG, S-PBOU, N-SEG and S-STE) reporting higher species richness when using eDNA (Fig. 3D). TEF reached similar yields (compared to eDNA) at one station (S-FR) and outperformed eDNA at three stations (S-DOR, N-FSJ and N-MROU) (Fig. 3D). TEF appeared to be more effective in detecting species such as *Xiphocaris elongata* (absent in eDNA at S-DOR, S-FR, N-FSJ, N-MROU and S-PBOU) and *Macrobrachium heterochirus* (absent in eDNA at N-BAS, S-DOR, S-FR, N-GUE and S-PBOU) (Fig. 3D). Inversely, eDNA was more effective in detecting certain

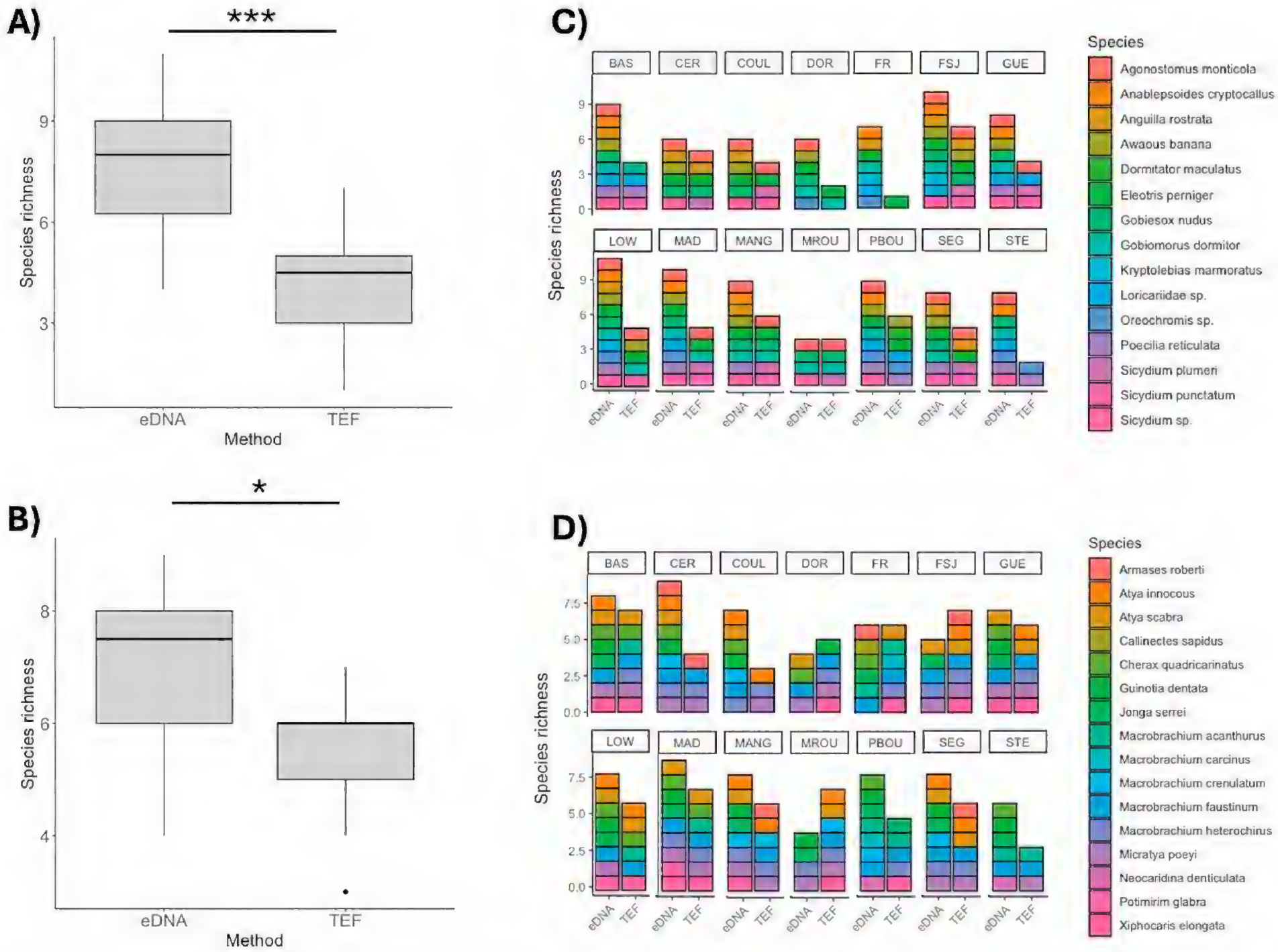

Figure 3. Comparison of species richness between the traditional electrofishing (TEF) and the eDNA methods, all stations combined for fish (**A**) and decapods (**B**) and then independently for each of the 14 stations studied, highlighting the species detected by both methodologies for fish (**C**) and decapods (**D**). * < 0.05 and *** < 0.001

hard-to-find species, such as *Jonga serrei* or *Neocaridina denticulata*, or highly invasive species *Cherax quadricarinatus* at station N-COUL (where it was unknown until now; Baudry et al. 2021) (Fig. 3D).

Biodiversity assessment using eDNA

Southern stations generally exhibited species-poor assemblages dominated by invasive taxa such as *Oreochromis* sp. and *C. quadricarinatus* (Fig. 4). For instance, S-STE (0.66 and 0.83, respectively for fish and decapods), S-PBOU (0.78 and 0.84, respectively for fish and decapods) and S-FR (0.37 and 0.86, respectively for fish and decapods) reported relatively low Simpson index values (see Suppl. material 1: S1). In contrast, northern stations harbored more diverse and evenly distributed communities (e.g., N-FSJ, N-BAS, mean 0.93 ± 0.02 for fish and 0.9 ± 0.01 for decapods) composed of native species like *A. monticola* or *Atya* sp. (Fig. 4). It was noted that some northern stations reported also low Simpson index values, such as N-COUL (0.53 for fish) or N-MROU (0.77 for decapods) (see Suppl. material 1: S1). Despite these apparent contrasts, also visualized through NMDS ordinations (Fig. 5), PERMANOVA tests did not show statistically significant influence of exposition for fish (R^2 = 0.05, p = 0.67), decapods (R^2 = 0.10, p = 0.19), or mixed datasets (R^2 = 0.08, p = 0.39), likely reflecting high within-group variability.

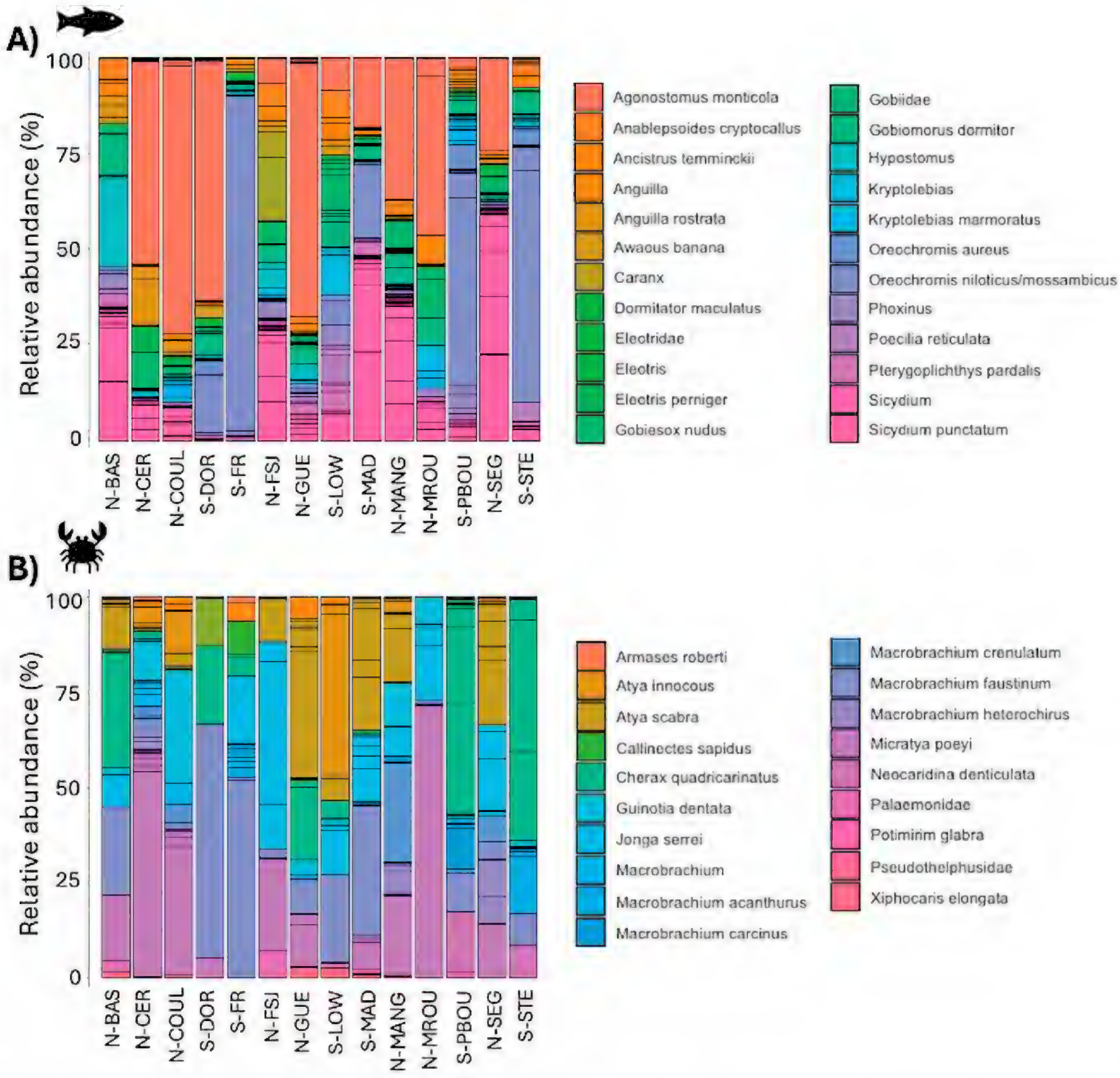

Figure 4. Fish (**A**) and decapods (**B**) taxonomy inventory reported in relative abundance of reads for the 14 studied stations, which served to calculate dissimilarities of community assemblages depending on the location (northern or southern of Martinique).

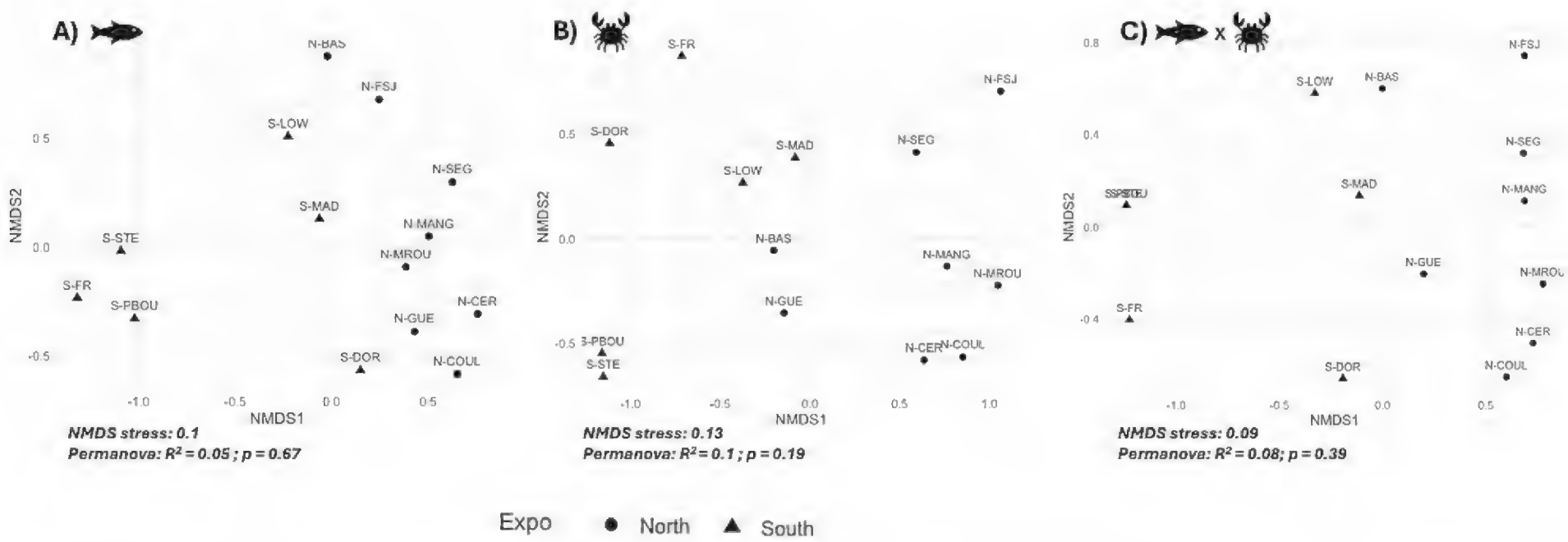

Figure 5. NMDS plots performed with the Bray-Curtis dissimilarity index calculated from the fish (**A**), decapods (**B**) and mixed taxa (**C**) eDNA-based datasets.

Conversely, RDA models highlighted significant relationships between environmental variables and community composition in all datasets. For all taxa (fish, decapods and mixed), the global RDA model highlighted the north/south exposition to be the most influential driver ($F = 5.69$, $p = 0.001$ for fish; $F = 2.93$, $p = 0.005$ for decapods; $F = 3.77$, $p = 0.001$ when combining fish + decapods) (Fig. 5). The temperature and conductivity parameters seemed also to influence significantly the fish communities ($F = 2.6$, $p = 0.03$ and $F = 2.39$, $p = 0.030$ respectively, in sequential tests) but their marginal effects were weaker ($F = 2.18$, $p = 0.07$; $F = 2.05$, $p = 0.06$, respectively) (see Suppl. material 1: S5). This influence of geographical exposition was supported by further analysis, showing a significant influence of geographical distance on communities' dissimilarities: for both fish (Mantel $R = 0.31$, $p = 0.02$) and decapods (Mantel $R = 0.52$, $p < 0.01$), nearby stations tend to host more similar communities.

Discussion

In this study, we evaluated the operationality of the eDNA metabarcoding approach as a reliable tool to monitor fish and decapods in Martinique, located in the seldom studied Caribbean region in terms of eDNA. Indeed, this study represents the second of its kind in this region, after Lefrancois et al. (2024) in Guadeloupe, and we showed very promising results with a view to implement this metabarcoding approach as a regular biomonitoring tool. We first compared the eDNA-based method and the traditional one (TEF), highlighting a higher number of species detected when using the first one, and a confirmation of most of the species already detected by TEF, especially for fish. However, we encountered some issues when it comes to discriminate genetically (with eDNA-based method, due to lack of taxonomic resolution in the database and/or the primers used) close species (such as *Sicydium* sp. or *Macrobrachium* species), possibly leading to a biased representation of some of them within the assemblage.

Validation of the eDNA-based metabarcoding method

The eDNA-based metabarcoding for fish detection has been now largely used, representing a major part of the studies led in the field of water biomonitoring (Belle et al. 2019) and giving rise to many different protocols and assay testings (Miya et al. 2015; Valentini et al. 2016; Macher et al. 2023). Here, we successfully implemented a multi-marker metabarcoding approach targeting both decapods and fish, achieving efficient detection of these taxa without apparent issues (e.g. inhibition, etc.). This is particularly noteworthy given that tropical freshwaters often present challenging conditions for eDNA surveys, such as high UV exposure, high water temperatures, probably inducing a quick DNA degradation rate. We highlighted a better reliability of eDNA-based method compared to TEF, with almost twice the number of species detected with eDNA (7.93 ± 1.94 species vs. 4.28 ± 1.68 by TEF) and most importantly in this validation context, a high coherence between the species caught with TEF. Nevertheless, we faced issues in using both methods, related to their inherent biases. For instance, TEF is an efficient method to capture individuals in the surrounding area (few m^2 around), but with reduced chances of capturing certain rare, cryptic species or those hiding under the banks. This trend was

observed in Guadeloupe, another Caribbean island close to Martinique, where Lefrancois et al. (2024) were unable to capture the hard-to-find species *J. serrei* (but detected by eDNA). Inversely, TEF was more efficient for *X. elongata* detection compared to eDNA-based method, probably due to the lower shedding rates which, as reported in a morphologically and genetically close species (the glass shrimp), estimated three times lower than those of fish, for example (see Allan et al. 2021 and references therein). Finally, the high morphological similarities between species from the same genera or family can lead to confusion during visual taxonomy assignments, for example in *Macrobrachium* sp. young adults, presenting underdeveloped rostrum or chelae – used for species determination (Lim et al. 2002). Such misidentification can also be encountered with Atyidae species, which include the natives *Atya innocous, A. scabra, Micratya poeyi, Potimirim* sp. and *J. serrei* but also the introduced *N. denticulata*, all characterized by a reduced size (around 20 to 40 mm, except largest *Atya* sp. males, reaching up to 100 mm) and an almost transparent color (Lim et al. 2002).

Concerning the eDNA-based method, it was not possible to confidently discriminate the two *Sicydium* species (*S. punctatum* and *S. plumieri*), despite many efforts to complete the database, probably due to the genetic similarity between these two species and with the closely related species *S. altum* (present in Costa Rica). This refinement of the database was shown to improve the biodiversity assessment in under-studied biodiversity hotspot like in Madagascar (Oliveira Carvalho et al. 2024), but in this latter study, the authors combined three 12S markers (MiFish, tele02 and 12S-V5). The use of several markers would be worth considering for future studies, even if here, the relatively small number of aquatic species (fish and decapods) present in Martinique (Lim et al. 2002) may have contributed to a detection of all of them as, or even more, effectively than with TEF. Concerning these *Sicydium* sp., the case of hybridization between these two very close species cannot be ruled out either, in which case metabarcoding is known to be unable to discriminate hybrids from parental species (Di Muri et al. 2020). That said, this issue does not represent a huge problem, as these two species have similar morphological characteristics and ecological functions (Lim et al. 2002), so the presence of one or the other makes little difference to the ecological state of the environment concerned. If specific monitoring of *Sicydium* sp. is needed, a targeted approach such as qPCR could be implemented to improve species discrimination and detection sensitivity. Finally, eDNA metabarcoding methodology appeared to have a considerable contribution when it comes to the early detection of invasive species, such as *Oreochromis* species or several Loricariidae species, initially with *Hypostomus robinii* known to be the only species in Martinique (Dubreuil et al. 2021) (more details below).

eDNA biodiversity assessment in Martinique

The eDNA-based metabarcoding method enabled to assess the ecological state of the stations studied, in a non-disruptive way, by calculating Simpson index and investigating the influence of stations' characteristics on species assemblages. Across the stations, a spatial structuring of aquatic assemblages was observed, with marked contrasts in community composition between northern and southern stations, also observed in TEF. In the southern rivers, community homogenization appeared frequent, with some stations dominated by a single taxon — particularly

the invasive *Oreochromis* sp. for fish and *C. quadricarinatus* for decapods. This dominance probably results from long-term effects of invasive species presence, particularly impacting in such fragile tropical island ecosystems, as for instance in Puerto Rico where 46 species were reported in freshwaters, with only 20% being native (Rodríguez-Barreras et al. 2020). Such dominance likely reflects a combination of biotic and abiotic factors facilitating invasions in these areas, including higher anthropogenic levels, inducing habitat modifications/disturbance and a higher propagule pressure (e.g. aquaculture, ornamental trade) (Cucherousset and Olden 2011; Baudry 2022). Inversely, the northern stations are often more preserved, sometimes characterized by very hilly landscape resulting in a torrential hydrographic network where the establishment of an invasive species is theoretically more complicated. As a result, these stations exhibited higher taxonomic evenness and harbored communities largely composed of native species such as *A. monticola*, *Atya sp.*, and *Macrobrachium sp.* — taxa quite widespread throughout the territory (Lim et al. 2002). However, these biodiversity assessment results should be interpreted carefully in such an environment. As an example, the N-MROU station seems to have quite bad results in terms of ecological state even though it represents one of the most preserved stations in Martinique, free of invasive species to date. It only suffers from a very particular, rugged ecosystem adapted to very few even native species (Lim et al. 2002). Finally, Simpson index is based on eDNA emitted by species with variable physiological characteristics and therefore variable eDNA shedding rates (Allan et al. 2021), biasing these calculations, especially in station populated mainly with decapods.

Beyond these community-level patterns, the eDNA-based approach enabled us to update occurrence records for several ecologically important species, including native taxa in decline whose last official surveys dated back many years (e.g., Lim et al. 2002), as well as certain invasive species. Thus, species such as *J. serrei*, reputed to be hard to observe due to its small size, or even *M. carcinus*, in decline in the territory, could be detected for decapods. It also confirmed the presence of the only endemic freshwater fish species of Martinique, *Anablepsoides cryptocallus*, on the N-GUE, N-BAS, S-STE, S-PBOU, N-STJ and S-FR stations (Baudry et al. 2023), but also to open up perspectives to its presence on additional stations (S-MAD and S-LOW). Interestingly, the *Kryptolebias marmoratus* cryptic species, living hidden in the mud (Taylor 2012), only known on a single station in Martinique (Baudry, Pers. Obs.), was detected here on two new stations (S-FR and N-FSJ) presenting plausible characteristics for its habitat (i.e. water with high conductivity and direct proximity to the mangroves). More worryingly, this study made it possible to expand the invasion zone of the well-known *C. quadricarinatus* (N-COUL) (Baudry et al. 2021) or to potentially highlight the occurrence of several invasive species of Loricariidae sp. on the territory instead of just one, *H. robinii* (Dubreuil et al. 2021).

Conclusion and implications for the future

Here, we confirmed the eDNA-based metabarcoding approach as a reliable tool for monitoring fish and decapods in Martinique, in the Caribbean, often considered as an understudied region. The present study, the second of its kind after Lefrancois et al. (2024) in Guadeloupe, showed very promising results for the implementation of this molecular method in regular biomonitoring programs,

confirming most of the species caught by TEF and revealing the presence of additional (native or sometimes more worryingly invasive) species. This metabarcoding method showed limitations in discriminating some genetically close species (e.g. *Sicydium* sp.), potentially leading to under-representation of communities' assemblages when it comes to calculate biodiversity indices, but not affecting the functional diversity in presence. That said, such metabarcoding data allowed to appreciate the ecological state of the stations studied, in a non-disruptive way, using different biodiversity indices (species richness, Simpson and Bray-Curtis), and to investigate how stations' characteristics (using NMDS, PERMANOVA and RDA analysis) shape assemblages across Martinique. Finally, even if the eDNA-based method offers the ability to early detect invasive species or the discovery of new suitable areas for endemic and/or rare native species, traditional methods, such as TEF, remain indispensable for certain aims, for example genetic studies, and both methodologies could be used in a complementary way. Harmonization of eDNA protocols is crucial to maximize the effectiveness of biodiversity studies and we encourage stakeholders to join such an initiative to shed light on the rich biodiversity occurring in poorly studied regions and to facilitate the fight against invasive species, one of the leading causes of biodiversity loss.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Use of AI

No use of AI was reported.

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Author contributions

TB: Conceptualization, Methodology, Data curation, Formal analysis, Investigation, Software, Validation, Visualization, Funding acquisition, Writing – original draft, Writing – review & editing. VV: Validation, Software, Supervision, Writing – review & editing. CD:

Investigation, Validation, Writing – review & editing. AA: Funding acquisition, Administration, Writing – review & editing. FR: Funding acquisition, Administration, Writing – review & editing. GL: Funding acquisition, Administration, Writing – review & editing. CMM: Funding acquisition, Administration, Writing – review & editing. FG: Conceptualization, Validation, Supervision, Funding acquisition, Administration, Writing – review & editing.

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Data availability

All data generated or analyzed during this study are included in this published article (and its supplementary information files), are accessible in Zenodo repository (doi: 10.5281/zenodo.17227561) and available from the corresponding author upon reasonable request.

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Supplementary material 1

Supplementary information

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Data type: docx

Explanation note: **S1.** Biotic and abiotic characteristics of the 14 stations studied. **S2.** Presentation of the use of mock communities, used as positive and calibration controls in the present study. **S3.** Output tables generated after using the packages phyloseq, ape, vegan and geosphere. **S4.** Number of reads generated with Illumina NextSeq 2000 and reads count after each curation step, for both decapods and fish and for each station. **S5.** Results of the redundancy analyses (RDA) investigating the effects of Exposition (North/South), Temperature, pH, Conductivity and Oxygen concentration on community assemblages (fish, decapods and both mixed).

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